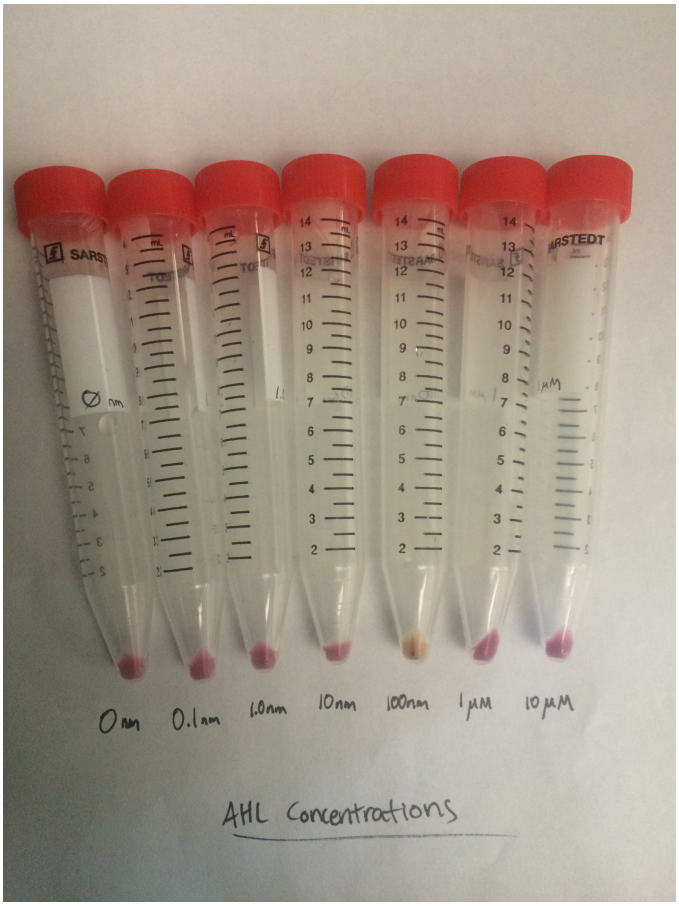
Title: Characterization of LuxPr promoter
Goal: Characterize the LuxPr promoter by measuring RFP fluoresence
Biobricks used in this experiment: BBa_F2620, BBa_J04450
Date range: 8/20/15 – 9/15/15
Members that worked on project: Hannah Young

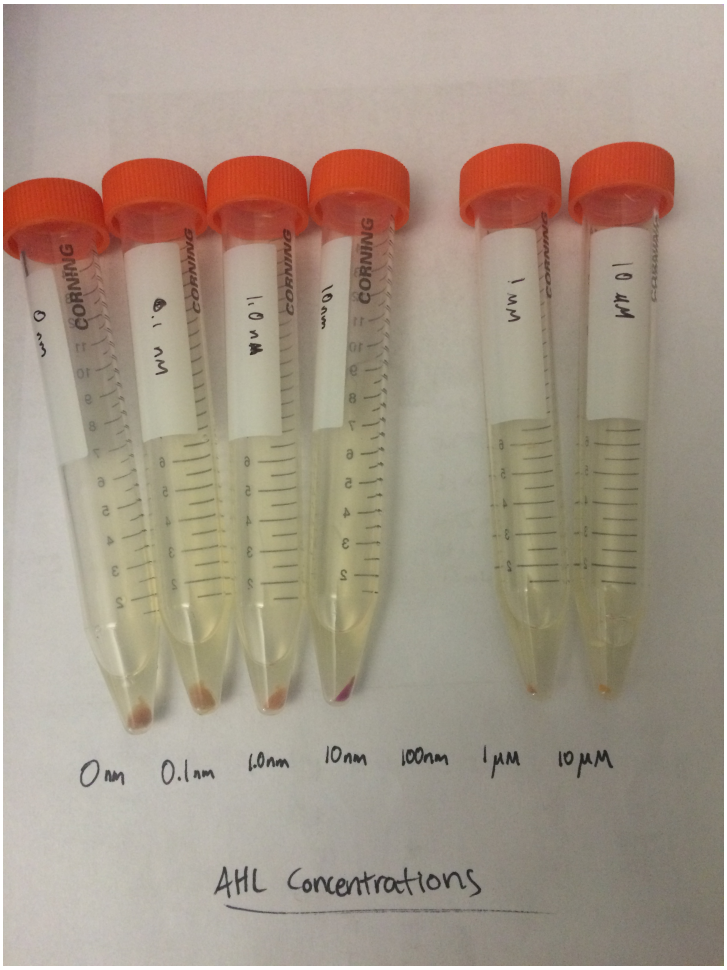
To characterize and test the leakiness of the LuxPr promoter, I created a construct that placed the LuxPr promoter in front of RFP on a 1C3 backbone. When induced with (N-(B-ketocaproyl)-L-homoserine lactone, a specific AHL molecule, the LuxPr promoter will allow transcription of RFP.

To characterize the promoter activity, we measured the RFP fluorescence with a flow cytometer. We tested cells grown in 5 mL LB or 5mL M9 media with 25uL chloramphenicol at different concentrations of AHL starting at 0.1nM, increasing by a factor of 10 (0.1nM, 1.0nM, 10nM, 100nM, 1uM, 10uM). We also included a negative control with no AHL added (0nM). These overnights were incubated for 18 hours, then centrifuged so the pellets could be compared in red color by eye.

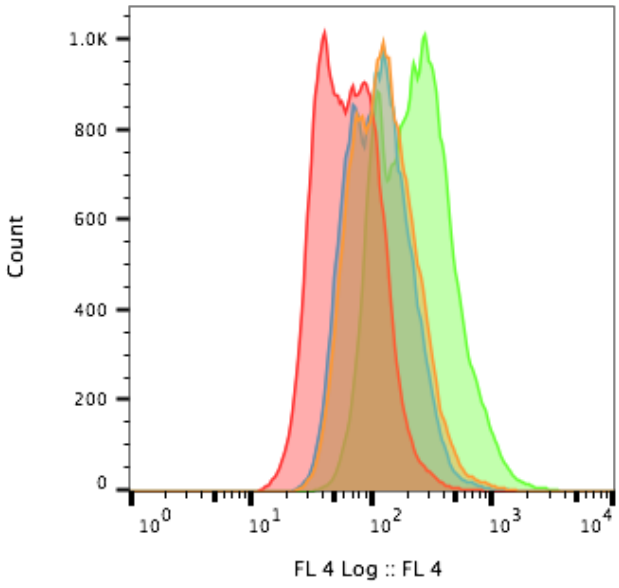
The first trial using LB media yielded all samples except 100nM turning a similar shade of deep red. This included the negative control turning red, giving us no baseline for comparison.



The second trial followed the same procedure as the first, but I replaced LB media with M9 media. However, there was no growth in any of the cells.
For the third trial, a different thiamine rich M9 media but still had minimal growth and no pellets.
One additional different M9 media was used for the fourth trial, and once again there was no growth.
The fifth trial was performed in LB. The 100nM sample did not grow.



There was a gradient of increasing red color between 0nM through 10nM, which appeared the reddest to the eye. The samples from trial 5 were suspended in PBS and then run through a flow cytometer to measure the relative fluorescence. Below is the cytometer data. 1H (pink) is the 0nm negative control. 2H (blue) is the 0.1nm sample. 3H (orange) is the 1.0 nm sample. 4H (green) is the 10nm sample.



	Sample Name	Subset Name	Count
	3H.fcs	Ungated	51504
	1H.fcs	Ungated	50739
	2H.fcs	Ungated	51916
	4H.fcs	Ungated	51202

Discussion

All three of the samples with AHL added produced relatively more fluorescence than the negative control. A concentration of 10nm produced the most RFP, so would be the ideal concentration to use according to this data. However, the upper concentrations we aimed to test failed to grow, so the experiment should be redone to determine if a higher concentration of AHL would be more optimal.

We had planned to test the promoter on a 4C5 backbone instead of the 1C3 used in this experiment. We hypothesized the lower copy number plasmid would provide more distinction between the concentrations of AHL, especially after our first result in which all concentrations including the negative control turned a deep red. The experience page with the promoter also described achieving good results using EZ media, an additional component we were not able to complete either.